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SOURCE Veterinariya, Vol XXVII, No 7, 1950.PRESERVATION AND RESTORATION OF THE VIRULENCE OF LEPTOSPIRA

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The preservation and restoration of the virulence of microbes and viruses is of great practical importance in immunology, epizootology, and epidemiology.

Virulence is not a stable property and many types of microbes quickly lose it when they are cultivated on artificial nutrient mediums, since the various protective agents of the host which have a harmful effect on the microbes are not present and the microbes lose their ability to defend themselves against these agents.

Although there are a number of methods which are successfully employed to reduce the virulence of microbes, it is considerably more difficult to increase their virulence. The most widely used method involves the passage of less virulent strains through susceptible animals in order to increase their ability to protect themselves against the antibodies of the hosts, but this does not always achieve positive results. We maintain that the difficulty in increasing the virulence on artificial nutrient mediums with the aid of specific antisera lies in the insufficient study of the dosage of the given agent, and that for this reason the method in question is not more widely used in laboratory practice.

In the process of diagnostic examination of the serum of animals with leptospirosis by the microagglutination-lysis reaction we noted the following phenomena; in high dilutions of the serum where lysis had not yet made its appearance and agglutination was expressed in a very weak degree, the leptospira evidenced increased activity and the number of them in the field of vision was considerably higher than in the control specimens.

On the basis of this observation, we decided to test the effect of a minimum quantity of specific antiserum on the virulence of leptospira cultivated on an artificial medium.

- 1 -

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50X1-HUM

Three strains of leptospira were selected for the test; the Strelok strain taken from sick horses (serological Type I), strain 148 taken from dead polar foxes (serological Type II), and the Kondrat'yev strain taken from humans (serological Type V).

The Strelok and 148 strains were taken from animals with leptospirosis by Lyubashenko and the author in 1946, and after cultivation on an artificial medium for 2 years had nearly lost their virulence. The Kondrat'yev strain made at the Institute imeni Mechnikov was also weakly virulent.

These three strains were cultivated on Uhlenget medium to which a very small amount of antiserum, not enough to inhibit their growth, was added. The dosage of antiserum depended on their activity.

Four different antisera were used in the test; one from rabbits immunized by strain 148, one from rabbits immunized by the Strelok strain, another from guinea pigs immunized by the Kondrat'yev strain, and the last from horses immunized by a mixture of the 148 and Strelok strains.

After a series of successive transplantations of the three strains in the subject medium, with the addition of the various antisera, their virulence was tested by infection of susceptible test animals.

Adult silver foxes were infected by the cultures of the Strelok and 148 strains, and guinea pigs (180-200 grams) by the Kondrat'yev strain.

As a control, animals were infected by these same strains which had not been treated by the antisera and by the same cultures which had undergone passage through animals.

The test results were as follows:

1. The control Strelok and 148 strains which had not been treated by the antisera did not cause disease in the six silver foxes they were used to infect, and the Kondrat'yev strain also exhibited weak virulence. Of two infected guinea pigs, one became sick and died on the 14th day.
2. The 148 strain which underwent passage through a silver fox and a ferret also exhibited weak virulence. Of two foxes infected, one became sick and died on the 17th day.
3. The strains treated with dissimilar antisera such as the Strelok strain (serological Type I), treated with 148 antiserum (serological Type II), or vice versa were found nonvirulent. Three foxes infected with the cultures did not develop the disease.

The Kondrat'yev strain (serological Type V), treated with the 148 antiserum increased in virulence. Two guinea pigs which were infected, developed the disease and died, one on the 6th and the other on the 9th day.

4. The strains treated by an antiserum produced as a result of the immunization of a horse by a mixture of strain 148 and the Strelok strain showed an increase of virulence. Three silver foxes infected by the Strelok strain became sick, and two died, one on the 10th and the other on the 20th day. The third recovered. Five foxes infected by strain 148 all developed the disease, four dying on the 6th or 7th day and the fifth recovering.

5. The strains treated serologically by a monotypic antiserum also exhibited an obvious increase in virulence. Five foxes infected by the Strelok and 148 strains all became sick and died on the 6th or 7th day. Two guinea pigs infected by the Kondrat'yev strain also became sick and died on the 6th to 8th day.

- 2 -

SECRET

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The data cited shows that strains of leptospira cultivated on an Uhlenhut medium to which specific antiserums have been added exhibit considerable increase in virulence. Besides this, it must be noted that in order to increase virulence of strains freshly taken from animals or which have undergone passage, only one or two transplantations on Uhlenhut medium with specific antiserums added are necessary, whereas old strains require many transplantations.

At the time of this investigation it was also noted that the strains treated by the antiserum exhibited more resistance to lysis and agglutination than the control strains. This increase in the resistance of the leptospira can be attributed to the selection of a more adapted and resistant type as a result of cultivation in the presence of antibodies of specific serums, in consequence of which they acquire new ability to adapt themselves to unfavorable surroundings.

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- 3 -

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